FORMULATION DEVELOPMENT AND EVALUATION OF PROPOLIS MOUTHWASH FOR TREATMENT OF PERIODONTAL DISEASE.

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ABSTRACT

Background: The aim of study is to formulate and assess the herbal mouthwash that possesses anti-inflammatory properties and is used in the treatment of periodontal disease. The chief ingredient of this formulation is propolis or bee glue obtained from the honeycomb.

Method: To assess the quality of the mouthwash, organoleptic features such as color, odor and appearance were evaluated. Chemical and physical characteristics such as pH, viscosity, bio burden, and irritancy test on the skin was carried out. Mouthwash was tested in the accelerated stability condition along with 40 °C / 75 % relative humidity and at room temperature (RT) for 6 months. At the end of the trial, participants were interviewed about the different aspects and outcome of the mouthwash to assess the level of acceptance by the participants.

Result: The resultant formulation is clear, brown and sweet. The final pH and viscosity of the mouthwash is 5.0 and 630cp respectively and none of the physical properties were altered at accelerated statibility temperatures.

Conclusion: The mouthwash is effective in the treatment of periodontitis and the composition is stable in accelerated stability conditions upto 6 months. No sign of irritation or bacterial growth is seen. When interviewed, participants responded with satisfactory remarks.

Keywords- Mouthwash, Propolis, Periodontal disease, Stability studies.

1 INTRODUCTION:

Periodontal disease is an infection-derived inflammatory disease, the prime causes of which are the presence of microbes and dental plaque at the margins of the gingiva (1). With the progression of the disease, destruction of periodontal ligament takes place resulting in deep pockets and a favorable ecological environment for microbes (2). As the disease progress to hit the severe stage, osteoclastogenesis is the most characteristic feature destroying alveolar bone and tooth-supporting structure (1). It is the leading cause of tooth loss (FDI World Dental Federation Report) and is accepted as a world health issue that affects 90% of the world population in milder form while severe form effect 20% of the world's population (3).

These deep pockets are inaccessible by instruments and ordinary brushing so scaling and root planning alone cannot eliminate the pathogenic bacteria. For this purpose the use of antimicrobial mouthwash is accepted as an ideal vehicle for chemical plaque control (4). Liquid mouthwashes that are commercially available contain synthetic active ingredients that cause teeth staining, xerostomia higher alcoholic content, taste disturbances and stability issues (5). All these adverse effects and drug resistance has sparked off interest to discover

the medicinal potential of herbs to treat periodontal pathologies consequently past decade has witnessed a surge in-vitro and in-vivo experiments on herbs (3).

Propolis has long been used for healing different oral conditions and its anti-inflammatory, antibacterial, antioxidative, antifungal, antiviral and antitumor properties have been proven (6). Egyptians were the first to used propolis as a medicine and Greek and Romans continued its use. Propolis is a bee product. It is a hard resinous substance consisting mainly of plant extracts and wax. It acts as protective agent in bee colony against invasion and infection, that seal the hive and providing the bees with an 'immune system'(7).

The complex chemical composition has made it difficult to standardize the beneficial outcome because it is too much dependent on the environmental condition, geographical location, type of plant pollen and bee species (8). For the very same reason, different shades of propolis are visible in the sample of propolis collected from different regions of Pakistan that may possess different biological properties (Figure 1).

Figure 1: Raw propolis before extraction.

This study aims at developing the mouthwash using this natural product to check its efficacy against the periodontal condition and evaluate its properties and assess its acceptability by patients.

2 METHODS:

Instrumentation:

Grinding machine (West point automatic series TSK 333 France), Lyophilizer (freeze dryer model FDI, Eyela, Tokyo Rikakekai co. Ltd Japan), Electronic balance (XT220AChina), Rotary evaporator (Eyela Japan), Viscometer (Dv-II+Pro Brookfield USA), pH meter (730 WTW-Germany), Stability chamber (KBF 720 Switzerland).

Chemicals:

Ethanol of purity 90%, Aspartam, citric acid, Methyl Paraben , Propyl paraben ,Glycerine, distilled water.

Collection and authentication of Material:

The propolis used in the preparation of the mouthwash is collected from the region of Thatta and Badin (Pakistan) during March and April 2021 from the crops of Clover (berseem) and Sunflower. The propolis collected was authenticated by the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi. Voucher specimen No. A00179 was deposited at the herbal museum of the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. Pakistan

Extraction of Material:

Propolis was washed with sterile water, shadow dried and stored in air-tight bottles. The hydroalcoholic extract was prepared by soaking and keeping it for 15 days. After 15 days extract was filtered and allowed to evaporate under controlled temperature (40ºC) and pressure through a rotary evaporator. The residues were lyophilized through a lyophilizer under reduced pressure. The final extract will be used for the formulation of mouthwash.

Figure 2: Propolis extract.

Formulation of mouthwash:

Mouthwash was formulated in three phases. In phase I Xanthan gum was triturated with glycerine, aqueous part was prepared by dissolving the required quantity of propyl and methyl paraben in boiling water at 70 ºC in phase II. Separately, dissolve aspartame in water and then add it to the aqueous part. Then add citric acid. In phase III tween 80 and 20% of the extract was added. Thereafter, all the three phases were combined by mixing them continuously to get a clear solution. The final volume will be adjusted to 100 ml by adding water as a solvent. The brown color mouthwash was prepared.

Evaluation of Propolis Mouthwash:

Accelerated stability test:

The efficacy, prompt actions and stability are the characteristics of an ideal mouthwash formulation. To maintain the physical, chemical, therapeutic, toxicological and microbiological properties, the formulation should be saved in inert container. Standard stability testing of the Propolis mouthwash was carried out and observed under ambient and accelerated conditions for 6 months according to the ICH guidelines.100 ml of Propolis Mouthwash was kept at ambient temperature (RT) room temperature and 100ml at 40 °C / 75 % RH in a stability chamber and noticed the mouthwash odor, color appearance, bioburden, pH and viscosity on 0, 1st, 3rd and 6th months. The organoleptic evaluation was carried out by odor, color, and appearance etc. The physical properties of mouthwash were evaluated by the following features.

pH:

100 ml of mouthwash was used to check its pH.

Viscosity:

100 ml mouthwash was used to find out the viscosity (cps) with spindle RV6 measured at 10 rpm.

Bioburden test:

Bio burden test is performed to determine the contamination in any given formulation, in this regard a TSA was performed. TSA agar has been used to determine aerobic bacteria count. Poured 1 ml of a 1:20 dilution of the material aseptically onto two sterile Petri dishes

labeled TSA. Then, 15-20 ml of melted tryptic soy agar (TSA) cooled to 40-45°C was applied to the plate. Mix the ingredients by swirling, then let them harden. These plates were incubated for 48 hours in the incubator at 30-35 degrees.

Skin Irritancy test:

A skin irritancy test was performed to check the allergic potential or any other adverse effect of the formulation i.e Propolis Mouthwash. It was then applied on the right hand in area of 1sq.cm and time was noted. Irritancy, edema and erythema were checked for up to 24 hours, 48 hours and 72 hours intervals of time respectively.

Acceptability of Mouthwash:

Assessment of the product acceptability was done using the methodology used by Cheng and Periera by taking interviews at the end of the intervention on a questionnaire (9, 10). Patients have to rate mouthwash in terms of excellent, good and bad for the items given in the chart below. Items that earned a score equal to or above 80% are considered as acceptable.

	Odor			Flavor			Color			Consistency			Application		Satisfaction		
	Ex	Good	Bad	Ex	Good	Bad	Ex	Good	Bad	Ex	GOOD	Bad	Difficult	Not difficult	Ex	Good	Bad
No. of patient																	
%																	

RESULT:

After repeated number of trials, the ingredients with their accurate percentage (%) that are required to formulate 100ml of mouthwash are enclosed in Table 1.

Table 1: Formulation of 100ml mouthwash

Ingredients	Formulation	
	Base%	Mouthwash%
Xanthan gum	0.2	0.2
Glycerine	15	15
Aspartam	0.05	0.05
Citric acid	0.1	0.1
Propyl paraben	0.15	0.15
Methyl paraben	0.2	0.2
Tween 80	2.5	2.5
Extract		20
D.I water	q.s	q.s

The organoleptic properties of the mouthwash are enclosed in table 2. When taken in a transparent glass container, the formulation appears shiny, brown and clear with no clues of phase separation.

Table 2: Organoleptic Properties of Mouthwash

Parameters	Mouthwash
Color	Brown
Odor	Sweet
Appearance	Clear

After the formulation, the propolis mouthwash underwent some physical parameters such as viscosity, pH, bioburden test and skin irritancy test. Both mouthwash and base were assessed at ambient room temperature and accelerated stability conditions i.e 40°C and 75% relative humidity. The mouthwash was assessed at the end of 1, 3 and 6 months and none of the physical properties were found altered at the end of 3 and 6 months (Table 3).

Table 3: Physical parameter of mouthwash of base (B) and mouthwash (M) at ambient and accelerated condition RT, 40ºC / 75%RH)

physical	**Ambient RT**								**Accelerated Stability Condition(40ºC / 75%RH)**							
	B				**M**								**M**			
T	**0M**	**1 M**	**3 M**	**6 M**	**0 M**	**1 M**	**3 M**	**6 M**	**0 M**	**1 M**	**3 M**	**6 M**	**0 M**	**1 M**	**3 M**	**6M**
C	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
O	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
A	--	--	--	--	--	--	--	-	--	--	--	--	--	--	--	--
W	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

M=Month T=Time C= Colour O= odor A=Appearance W= weight RT =room temperature

-- No change -+ slight change ++ degradation

The final pH of the mouthwash is 5.5 at room temperature and reduced to 4.1 at accelerated stability conditions (Table 4). The viscosity of mouthwash is 630cp and 500cp at ambient and accelerated temperatures respectively (Table 5).

Table 4: pH value of base (B) and mouthwash (M) at ambient and accelerated condition RT, 40ºC / 75%RH)

Time	Ambient RT		Accelerated Stability Condition(40ºC / 75%RH)	
	B	**M**	**B**	**M**
0M	5.5	5.3	5.5	5.3
1M	5.3	5.1	5.2	4.4
3M	5.0	5.1	5.0	4.1
6M	5.1	5.0	5.1	4.1

M=Months

Table 5: Viscosity of base (B) and Mouthwash (M) at ambient and accelerated condition RT, 40ºC / 75%RH)

Time	Ambient RT		Accelerated Stability Condition(40ºC / 75%RH)	
	B	**M**	**B**	**M**
0M	500cp	666cp	500cp	666cp
1M	500cp	600cp	550cp	550cp
3M	550cp	650cp	500cp	50cp
6M	500cp	630cp	560cp	500cp

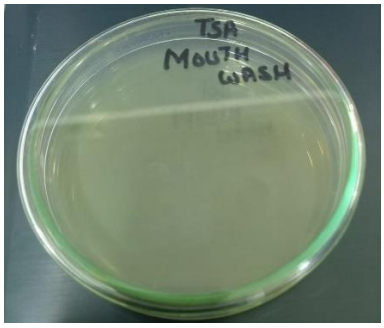
M=Months

No bacterial growth is seen at the end of the study in both base and mouthwash when kept at room temperature or in accelerated stability conditions which ensures the safety of the product up to 6 months of formulation (Table 6, Figure 3).

Table 6: Aerobic bacterial count of base (B) and mouthwash (M) at ambient RT and accelerated temperature 40ºC 75%RH

Time	Ambient RT		Accelerated Stability Condition(40ºC / 75%RH)	
	B	**M**	**B**	**M**
0M	Nil	Nil	Nil	Nil
1M	Nil	Nil	Nil	Nil
3M	Nil	Nil	Nil	Nil
6M	Nil	Nil	Nil	Nil

Fig 3: TSA test of mouthwash



No signs of erythema, allergy or any skin reaction appeared after the mouthwash is tested on the skin and left for up to 72 hours (Table 7).

Table 7: Skin Irritancy Test.

Skin Irritancy Test	Results
24 hours	No irritation persists.
48 hours	
72 hours	

At the end of a 3-month clinical trial, all 20 participants were interviewed based on a questionnaire about the odor, flavor, color, consistency, application and satisfaction with the product. As for the odor 85% (n=17) participants consider it good and 15 % (n=3) consider it bad. For flavour 80% (n=16) rated it good and 20% (n=4) rated as bad. 65% (n=13) participants find the color pleasant while 35% (n=7) rated as unpleasant. None of the 20 patients complain about consistency and application difficulty. All the participants were satisfied with its outcome in the treatment of periodontal disease. 45% (n=9) rated the product as good and 55% (n=11) marked it as excellent. Few patients reported slight burning in the mouth which subsided in a couple of minutes.

Table 8: Evaluation of the propolis mouthwash through patients interview.

	Odor			Flavor			Color			Consistency			Application		Satisfaction		
	Ex	Good	Bad	Ex	Good	Bad	Ex	Good	Bad	Ex	GOOD	Bad	Difficult	Not difficult	Ex	Good	Bad
No. of patient	-	17	3	-	16	4	-	13	7	-	20	-	-	20	11	9	-
%		85%	15%		80%	20%		65%	35%		100%			100%	55%	45%	

EX= Excellent

DISCUSSION:

Propolis is naturally obtained by the bee Apis Mellifera with more than 300 compounds that hold medicinal potential such as, phenolic compounds, essential oils, aromatic acids, amino acids and waxes. However, flavonoids account for their anti-inflammatory properties (11).

Stability is the most essential and crucial parameter for all drug preparations. It was concluded that the mouthwash would be stable at both room temperature or ambient conditions, with no colour, odour, appearance, pH, viscosity, or bioburden degradation occurring during a study at 40°C/relative humidity of 75%, which did not alter the effectiveness of the mouthwash. This also reflects a drug's compatibility with all excipients as shown in the table.

This mouthwash formulation was very well accepted and tolerated by the participants except for a few who found the color and taste of mouthwash a bit unpleasant. These findings coincide with those of Noronha et al. This issue arose because no coloring or flavoring agent was used in the process. These properties can be improved in future studies to increase the compliance of drugs (12).

For the acquisition of maximum efficacy and safety, the formulation was prepared according to the WHO guidelines which are necessary for the standardization of any herbal product followed by validation through pharmacopeia standards before the clinical trials.

CONCLUSION:

The formulation turned out to be effective for the treatment of periodontal disease. The composition is also found to be stable up to 6 months when tested in ambient and accelerated temperatures. No sign of irritation or bacterial growth is seen. Also this first time prescribed formulation earned great level of acceptance by the participants when interviewed at the end of the trial which ensures better drug compliance.

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CONFLICT OF INTEREST:

The authors have no conflict of interest to declare.

ETHICS APPROVAL:

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PATIENTS CONSENT:

Informed consent was taken from patients. Patient identity was not disclosed at any point during the research.

AUTHOR'S CONTRIBUTION:

SMW conceptualized and assist in lab work and wrote the manuscript, AH conducted the whole lab work, AR overall supervised and proofread the manuscript, FS helped in writing the manuscript, SSQ supervised the drug trial on patients, JAQ helped in literature search and data analysis.

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